

## Effect of Fertirelin Acetate or Buserelin on Conception Rate at First or Second Insemination in Lactating Dairy Cows

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### ABSTRACT

The objective of this study was to determine if fertirelin acetate and buserelin, two GnRH agonists, improve conception rate when administered at the time of first or second AI in lactating dairy cows. The study consisted of a common protocol conducted at 10 commercial dairy farms. Approximately 150 cows within each dairy were assigned randomly in replicates to receive intramuscularly either no injection or injection of 25, 50, 75, or 100 µg fertirelin acetate or 10 µg buserelin immediately after AI. Cows were subjected to the reproductive management practices normal for each location. Cows at each location were palpated for pregnancy status at 35 to 60 d postinjection. No improvement in conception rate was detected in response to either agonist (control = 48%, fertirelin acetate = 41.5%, buserelin = 39.7%). Conception rate was unaffected by either days postpartum at injection or overall fertility of the individual herds. These observations do not support the routine use of doses of 25 to 100 µg fertirelin acetate or 10 µg buserelin at the time of first or second AI as a means to improve conception rate in lactating dairy cows.

(Key words: gonadotropin-releasing hormone, fertirelin acetate, buserelin)

### INTRODUCTION

Administration of synthetic GnRH or its agonists (fertirelin acetate, buserelin) at or near the time of first or second AI has been reported

to increase (2, 5, 6, 7, 9, 10, 11, 13, 14, 18) or have no effect (1, 8, 12, 15, 20, 21) on conception rate in lactating dairy cows. Of the five studies conducted in the United States (10, 12, 15, 20, 21), four reported no effect of 100 µg GnRH on conception rate relative to conception rate of contemporary controls (12, 15, 20, 21). However, studies conducted in Japan, New Zealand, and Europe showed improvements in conception rate in response to GnRH, fertirelin acetate, or buserelin (2, 5, 6, 7, 9, 11, 13, 14). The reason for this divergent response from various geographical regions is not obvious.

The objective of this study was to determine if doses of 25 to 100 µg fertirelin acetate or 10 µg buserelin benefit conception rate when administered at the time of first or second AI in lactating dairy cows.

### MATERIALS AND METHODS

The experiment was conducted at 10 commercial dairy farms located in Washington, California, Arizona, Missouri, Florida, and New York. The experimental protocol was supervised by either the herd health veterinarian (8 dairies) or the full-time herdsman (2 dairies). Number of cows in milk at the dairies ranged from 306 to 3320. The study was conducted from June 1987 through February 1988. In states with warmer climates (CA, AZ, and FL) the study was initiated in late October 1987.

### Animals

To be eligible for the study, dairy cows had to have met each of the following criteria: 1) be lactating, 2) have been inseminated no more than once during the current lactation, 3) not been assigned previously to the study, and 4) been defined as eligible for breeding at the dairy. Therefore, only cows being inseminated

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TABLE 1. Analysis of variance of days postpartum at assignment to experimental group.

Source of variation	df	MS	P
Herd	9	18197.91	<.01
Block (herd)	238	1046.82	.04
Dose	5	328.84	.80 <sup>1</sup>
Herd × dose	45	704.04	.83
Error	1169	881.33	

<sup>1</sup>Testing term was herd × dose.

for the first or second time during the current lactation were included in the study. Cows that were treated at their first AI and returned to estrus could not be reassigned to the study. The intent of the protocol was to allow each dairy to use its routine reproductive management system, i.e., observation of estrus and AI techniques were consistent with management practices of each herd. Estrus and AI dates were recorded. All cows were palpated per rectum by the herd veterinarian to determine pregnancy status at 35 to 60 d posttreatment.

#### Experimental Drugs

Fertirelin acetate (des-Gly<sup>10</sup>-GnRH-ethylamide; fertirelin acetate discovered, licensed, and manufactured by Takeda Chemical Industries, Ltd., Osaka, Japan) is a nonapeptide with molecular weight of 1213.36 and potency three to six times that of GnRH in rats (4, 16, 22) and cows (J. R. Chenault; unpublished observation) as measured either by concentrations of serum LH and FSH or by induction of ovulation. Fertirelin acetate was supplied by the manufacturer as a sterile solution at a concentration of 50 µg/ml saline in 2-ml ampules.

Buserelin (des-Gly<sup>10</sup>-D-Ser (Bu)<sup>6</sup>-GnRH-ethylamide) was supplied in 10-ml vials as Receptal® (Hoechst Animal Health, Bucks, UK; 4 µg buserelin/ml) and was purchased from a veterinary supply house in the United Kingdom.

#### Experimental Groups

Doses administered were 0 (no injection), 25, 50, 75, and 100 µg fertirelin acetate and 10 µg buserelin immediately following AI by intramuscular injection. Except that no injection

was administered, the cows in the control group (0 dose group) were treated identically to cows in the other experimental dose groups. Only one dose was used from each ampule of fertirelin acetate, and the unused solution was destroyed.

The experimental dose groups were investigated contemporarily in each of the 10 dairies. Each dairy was provided with animal assignment sheets containing a listing of the six experimental groups that had been randomized within 25 blocks (each block consisted of the six experimental groups in random order). As cows were observed in estrus, and if they met the criteria for eligibility, they were assigned the next available line on the animal assignment sheet. In 9 of the 10 dairies, 25 cows were administered each dose. At the remaining dairy, 20 cows were administered each dose. The number of cows completing the trial and included in the statistical analysis were 243, 240, 242, 240, 242, and 240 for the 0, 25, 50, 75, and 100 µg fertirelin acetate and the 10 µg buserelin dose groups, respectively.

#### Statistical Analyses

To test for possible biases in assignment of cows to experimental groups, the distribution was plotted of cows per experimental group treated from either 0 to 60, 61 to 120, or greater than 120 d postpartum. In addition, differences in days postpartum at time of treatment were tested by an overall F test in an ANOVA (Table 1) using the GLM procedure of SAS (17).

The primary variable of interest was conception rate of the estrus at which treatment was administered, which was calculated as (number of cows pregnant ÷ number of cows inseminated) × 100 for each experimental group at each location. Conception rate data were transformed using the Freeman-Tukey arcsin transformation to ensure homogeneity of variance (3). Transformed data were analyzed by ANOVA as shown in Table 2 (17). The model included dose (as a fixed effect), herd (dairy, as a random effect), and dose × herd. Dose × herd was used as the testing term for dose effects. The dose degrees of freedom were partitioned into linear, quadratic, and remainder components to identify minimal and maximal effective doses of fertirelin acetate for improvement in concep-

TABLE 2. Analysis of variance for the Freeman-Tukey transformed conception rate data.

Source	df	MS	P
Herd	9	136.44	
Dose <sup>1</sup>	5	33.31	.40
Linear	1	117.54	.97 <sup>2</sup>
Quadratic	1	14.46	.75 <sup>2</sup>
Remainder	2	5.21	.85 <sup>3</sup>
Dose × herd	45	31.60	

<sup>1</sup>The buserelin experimental group was not included in tests for linear, quadratic, and remainder dose trends.

<sup>2</sup>One sided test using dose × herd as testing term.

<sup>3</sup>Two sided test using dose × herd as testing term.

tion rate (Table 2). Because the hypothesis of interest was an improvement in conception rate, the linear and quadratic dose trends were tested using one-sided tests. The buserelin experimental group was not included in tests for linear, quadratic, or remainder dose trends. All experimental groups were compared with the buserelin experimental group using least significant differences (LSD option of SAS).

To gain additional information from these data, several ancillary analyses were conducted. In general, these analyses were prompted by observations or trends mentioned in other published reports and were conducted in addition to those identified in the study protocol. To examine the effect of days postpartum at treatment on conception rate response to fertirelin acetate and buserelin, the data were divided into cows treated on or after 60 d postpartum (late) versus before 60 d postpartum (early) and each data set was analyzed by ANOVA. Duncan's multiple range analysis was used to test treatment effects within the early and late postpartum groups.

To determine the affect of fertility on conception rate response, the overall conception rate was calculated for each herd, and this value was used to divide the dairies into either a low fertility group (five herds with conception rates of 31.5 to 37.6%) or a high fertility group (five herds with conception rates of 47.7 to 55.4%).

## RESULTS AND DISCUSSION

There was no indication of bias associated with days postpartum at assignment to experimental group as evaluated either by visual ap-

praisal of the distribution of cows by days postpartum within experimental groups or by ANOVA of the days postpartum (Table 1). The dose by herd interaction for days postpartum at treatment was not significant ( $P=.83$ ) and no differences were detected in days postpartum at treatment across experimental groups ( $P=.8$ ; Table 1). Days postpartum at treatment were not different across experimental groups and were not analyzed further. The percentage of cows assigned to experimental groups at d 0 to 60, 61 to 120, and >120 postpartum was 26.5, 66.6, and 6.9, respectively. The overall mean was 78.7 d postpartum on the day of treatment. Herd had a significant effect on days postpartum at assignment to experimental group (Table 1). The herd effect most likely is attributed to the variability that existed between dairies in the voluntary waiting period before cows were defined as eligible for breeding. Block within herd also was significant (Table 1). The experimental protocol allowed cows to be assigned at either first or second AI. However, cows could only be assigned once to the study. This procedure for assignment to study resulted in many second service cows (with associated longer postpartum intervals) being assigned early in the study at each location whereas few second service cows were assigned late in the study. This assignment to study procedure seems to account for the significant block effect.

No trends were detected in the dose of fertirelin acetate conception rate response (linear or quadratic; one-sided tests;  $P>.75$ ; Table 2) nor could it be concluded that lack of fit of dose responses to the linear and quadratic curves was significant ( $P=.85$ ). Similarly, the main effect of dose was not significant ( $P=.40$ ). Using pairwise comparisons (Table 3; LSD; two-sided), no differences in conception rate responses were detected between cows injected with 10  $\mu$ g of buserelin and any other experimental group. Therefore, evidence is not sufficient to conclude that either fertirelin acetate or buserelin, at the doses investigated, injected at the time of first or second AI increased conception rate of lactating dairy cows.

In multiple location studies, location is expected to be a major source of variation. In this particular study, location accounted for the largest proportion of variation in conception rate of any source included in the statistical model (Table 2). This variation could be due to

TABLE 3. Least squares means for Freeman-Tukey arcsine transformed and actual conception rate data.

Experimental group	No. herds	Conception rate	
		Transformed (')	Actual (%)
No injection	10	44.0	48.2
Fertirelin acetate			
25 µg	10	41.2	43.6
50 µg	10	40.1	41.4
75 µg	10	40.2	41.6
100 µg	10	39.1	39.4
10 µg Buserelin	10	39.2	39.7
LSD <sup>1</sup>		5.1	...
Scheffé's LSD <sup>2</sup>		8.8	...

<sup>1</sup>Least significant difference (LSD option of SAS; two sided;  $P < .05$ ).

<sup>2</sup>Two-sided;  $P < .05$ .

any or all of the following: skill of technicians performing AI, length of the elective postpartum rest period, fertility of semen used, and criteria used to determine estrus utilized to inseminate cows.

Visual appraisal (Table 3) of the conception rate data indicated that all injected groups had conception rates numerically lower than the uninjected control group. Furthermore, conception rates of cows injected with fertirelin acetate appeared to decline slightly as dose increased. This possible trend in conception rate response to fertirelin acetate is in the opposite direction as hypothesized and, hence, was not able to be tested because of the a priori assumption that a one-tailed ANOVA should be used in the dose titration analysis. To pursue this observation further, the transformed conception rate data were subjected to comparisons using Scheffé's Test for a postpriori result (19). Using this analysis, no differences in conception rate were detected between any of the experimental groups (Table 3).

Sixteen published papers reporting studies in which either GnRH or a GnRH agonist was injected at the time of first AI in lactating dairy cows are cited in this manuscript. In these studies, conception rate response to treatment varied from -2 to +13.7 percentage points different from the conception rate of contemporary controls. In the study reported herein, the mean conception rate of experimental groups varied from 4.6 to 8.8% below that of uninjected

controls (Table 3). Review of these studies reveals few clues as to the reason for this range in conception rate response to GnRH treatment. Across studies, control cows were either injected with a placebo (2, 10, 11, 12, 13, 14, 15, 20, 21) or not injected (1, 6, 7, 8, 9, 18). From these reports, the conception rate response to GnRH or its agonist does not appear to be influenced by the type of control utilized.

Of the papers reporting little or no change in conception rate, all utilized native GnRH. In four of these studies, 100 µg GnRH was injected (12, 15, 20, 21). This dose of GnRH, in general, is low relative to either the dose of GnRH or potency of the GnRH agonists used in studies reporting a positive effect of treatment on conception rates. However, in two studies, 100 µg (10) or 125 µg (18) GnRH were reported to increase conception rate. Doses of 250 µg to 1000 µg GnRH have been reported to have either little to no effect (1, 8) or a positive effect (5, 6, 7) on conception rate. Similar discrepancies in conception rate response are now evident for fertirelin acetate. Nakao et al. (14) reported increased conception rates (+7.5%;  $P < .05$ ) following injection of 100 µg fertirelin acetate in a trial conducted in numerous herds under a clinical protocol in Japan. In the study reported herein, fertirelin acetate at doses of 25 to 100 µg had no effect on conception rates. Therefore, neither dose nor chemical structure can account for the range in conception rate response that has been observed following administration of GnRH or its agonists at first or second AI in lactating dairy cows.

In three recently reported studies (12, 15, 20), 100 µg GnRH injected at the time of AI had no effect on conception rate. Those studies were tightly controlled and conducted in the US in research herds (3 university herds and 2 USDA herds). The study reported herein was tightly controlled and conducted in 10 well managed, commercial herds. The additional similarity was that no beneficial effect of either fertirelin acetate or buserelin was demonstrated on conception rate when these were injected at the time of AI in lactating dairy cows. These results argue against recommending the routine use of either 100 µg GnRH, 25 to 100 µg fertirelin acetate or 10 µg buserelin at the time of first or second AI in dairy cows. Use of GnRH agonists at the time of AI may prove

beneficial at individual locations or under specific environmental or management conditions; however, the precise conditions under which GnRH or agonists may prove beneficial are not readily apparent from the existing published data.

Three studies have reported a greater benefit in conception rate response in cows treated with GnRH (5, 6) or fertirelin acetate (14) early in the postpartum period than later. In the study reported herein, no dose trends in the conception rate response were detected within cows treated either early (<60 d) or late ( $\geq$ 60 d) postpartum. No differences in conception rate were detected ( $\alpha=.05$ ; Duncan Multiple Range Analyses) between dose groups within stage postpartum. Mean conception rates were similar between cows treated before 60 d postpartum (44.1%) versus cows treated on or after 60 d postpartum (43.4%). These observations do not support the concept of increased response to GnRH agonists in cows treated earlier in the postpartum period.

Nakao et al. (14) reported an increase in conception rate in response to fertirelin acetate in herds with relatively low fertility (45.1% first service conception rate), whereas in herds with high fertility (56.5% first service conception rate), little or no response in conception rate to fertirelin acetate was observed. Overall fertility of the herds in the study reported herein had no effect on the conception rate response to either fertirelin acetate or buserelin. In low fertility herds, the mean conception rates of the uninjected controls and all injected cows were 41.8 and 33.3%, respectively (change = -8.5). In high fertility herds, the mean conception rates in uninjected controls and all injected cows were 54.7 and 48.8%, respectively (change = -5.9). This observation is not consistent with the concept of a greater response to fertirelin acetate in herds with lower fertility.

### CONCLUSIONS

The observations of no improvement in conception rate of either fertirelin acetate (25  $\mu$ g to 100  $\mu$ g) or 10  $\mu$ g buserelin and similar observations reported elsewhere following administration of 100  $\mu$ g GnRH (12, 15, 20) support the conclusion that the routine use of these GnRH agonists at the time of first or second AI for improvement of conception rate in dairy cattle

in the US cannot be supported. Conception rate response to fertirelin acetate and buserelin was unaffected by days postpartum when these agonists were administered. Therefore, the data fail to support the concept that there may be specific time periods during the postpartum period when administration of fertirelin acetate or buserelin would be more or less beneficial. Conception rate response to fertirelin acetate or buserelin was not influenced by fertility within the herd. No beneficial effect was observed in herds with relatively high or low fertility.

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